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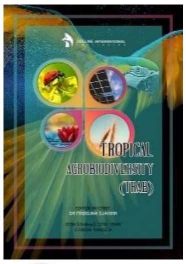

RESEARCH ARTICLE

EFFECTS OF SEED PRIMING ON SEED GERMINATION AND EARLY SEEDLING GROWTH OF CHILI (*CAPSICUM ANNUM* L.) UNDER WATER DEFICIT CONDITION

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ABSTRACT

Seed priming may improve seed germination and early seedling growth of plants under water deficit condition. The present study was conducted to investigate the effects of seed priming on the germination and early seedling growth of chili under water deficit condition and to identify the optimum rate of seed priming for higher seed germination and early seedling growth. The water deficit stress level towards early seedling growth also been identified. The priming treatments involved were non-priming as control, and priming with gibberellic acid (GA_3) at 5 mg/L, 10 mg/L and 15 mg/L. Seed germination experiment was conducted using wet-tissue method for seven days duration. Germination percentage, germination index and seedling vigour index were recorded in the seed germination experiment. The germinated seeds were then sown and grown under five different levels of water deficit treatment in accordance with the frequency of watering mainly watering 8 times (S1), 4 times (S2), 2 times (S3), once (S4), and not watered (S5), in a duration of 14 days. The early seedling growth performance was based on plant height, and root length. Seed germination and early seedling growth performance of 5 mg/L GA_3 primed seeds was significantly better as compared to other seed priming treatments. In addition, the S1 and S2 could be regarded as non-stress condition, S3 and S4 as mild to moderate stress and S5 as severe stress since all seedlings were not survived under S5 condition. In general, S2 was found to be the optimum watering treatment as it recorded the highest plant height and root length of the seedlings.

KEYWORDS

Seed Priming, Germination, Chili, Water Deficit

1. INTRODUCTION

Chili (*Capsicum annum* L.) is known to be one of vegetable crops which have various uses in culinary preparations and traditional medicine. It is a member of the Solanaceae or also known as night shade family that also consist of other common species in Malaysia such as tomato, potato, and petunias. The variety of this species can be characterized according to the different in sizes and shapes. This species is known to be vegetable that low in calories and high in potassium, vitamin A, and vitamin C content (Chew, 2018). Furthermore, fresh chili is proven to be an outstanding source of metabolites with renowned antioxidant activity which made it to have potential on treating disease against cancer, precluding from gastric ulcer and activating immune system (Materska and Perucka, 2005; Sun et al., 2007). All the beneficial characteristics of *C. annum* shows how important the crop whether in term of uses, nutritional status, or medicinal properties.

Hence, the high yield production of this crops is important, but it may encounter several obstacles that reduce the quality and yield of the crops. Seed germination and seedling growth may be affected by natural stresses such as water deficit. This problem can be overcome through several techniques that involve the treatment of the seed before sowing it into the soil. One of the techniques is seed priming that involves in hydrating the seed in numerous ways. Seed priming can be defined as pre-sowing treatment that will expose the seed to specific solution which allows partial hydration, and not germination, to occur (Heydecker et al., 1973). This method proven to improve germination rate, uniformity in emergence and germination of the crops under a wide range of environmental climates and improve the seedling vigor and growth (Venkatasubramanian and Umarani, 2010).

Through this technique, the metabolic activities which needed for the radicle protrusion may be activated (Heydecker et al., 1973; Passam and Kakouriotis, 1994). Common priming technique usually involve hydropriming, osmopriming, halopriming, and priming with plant growth hormones (Divya and NirmalaDevi, 2015). However, the effects of seed priming may varies depend on the priming reagents used, crops that been selected and natural stresses that they undergo. Water deficit has been regarded as one of the global issues towards agricultural crops including chilies (Jaleel et al., 2007). In fact, agricultural activities account for about 70% of total freshwater consumption (Schulte et al., 2011).

The climate change phenomenon such as El-Nino is also jeopardizing the availability of fresh water for human and agricultural consumption. In 1998, severe drought in certain areas of Malaysia caused water supply for irrigated agriculture to deplete due to low stream flow (Ahmad and Low,

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2003). According to a study, about 526.7 mm of water per plant is required over the grow season of chili (Owusu-Sekyere and Twum, 2001). Theoretically, if the amount of water consumption for such purposes could be reduced, we could increase the availability of fresh water for other beneficial purposes. In addition, such practice may also reduce farmer's incurred cost for irrigation. Hence, the present study was conducted as an attempt to explore the potential of seed priming in improving seed germination and early seedling growth of chili under reduced irrigation and water deficit condition.

2. Methodology

2.1 Experimental Design and Treatments

The study was conducted at the Laboratory of Plant Science and the Glasshouse and Nursery Complex (GNC), Kulliyyah of Science, International Islamic University Malaysia (IIUM) Kuantan Campus, Pahang. Chili seeds were procured from the Malaysian Agricultural Research and Development Institute (MARDI) located at Klang, Selangor. Germination percentage of the seed lot was tested and recorded following method (ISTA, 2016). The experiment was conducted using the Randomized Complete Block Design (RCBD) with three replications following (Salleh et al., 2018). Seed priming treatments involved were priming with plant growth hormone namely the Gibberillic acid (GA_3). The seed priming treatments were: T1) 5 mg/L GA_3, T2) 10 mg/L GA_3, T3) 15 mg/L GA_3 and T0) non-primed seeds as control of the experiment. The water deficit treatments were based on the frequency of watering in 14 days. The watering frequency were reduced to 8 times (S1), 4 times (S2), 2 times (S3), 1 times (S4), and none (S5) to induce water deficit condition. The frequency and interval days of watering in 2 weeks for each treatment as shown in Table 1.

Table 1: The water deficit treatment

Treatment	Watering frequency in 2 weeks	Interval days of watering
S1	8	2 days
S2	4	4 days
S3	2	7 days
S4	1	14 days
S5	0	0 days

2.2 Seed Priming Procedure

All seeds were soaked in their respective priming treatment for 24 hours, re-dried to their original moisture content of about 10±1% using the mechanical drying method at 35 °C following procedures (Salleh et al., 2020a). Seed germination test using wet-tissue method was then conducted for a total number of 2500 seeds for each treatment for 7 days.

2.3 Planting Procedure and Water Deficit Treatment

After 7 days of germination test, a total number of 50 germinated seeds per replication of each treatment were sown in planting tray containing peatmoss soil. All seeds were placed approximately 1.0 cm depth from the base of the planting tray. Then, another 1.0 cm of soil covered up the seeds that have been planted. Water was withheld to induce water deficit stress in accordance with their designated water deficit treatment.

2.4 Data Collection

The number of germinating seeds was observed daily and the germination percentage (GP) was calculated on day seven of germination test following formula (ISTA, 2016):

$$Germination\ Percentage = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds}\ x\ 100\%$$

In addition, the germination index which denotes speed of seed germination was calculated as described by the Association of Official Seed Analysis (AOSA, 1983).

$$GI = \frac{Number\ of\ germinated\ seed}{Days\ to\ first\ count} + \cdots + \frac{Number\ of\ germinated\ seed}{Days\ to\ final\ count}$$

The seedling vigour index was computed following (Abdul-Baki and Anderson, 1973).

$$SVI = seedling\ length\ (cm) \times germination\ percentage$$

The plant height was measured using ruler that have a precision of 1 mm on day 7 and day 14 after sowing. In addition, root length seedling was also measured on day 14 after sowing.

2.5 Statistical Analysis

All data were analysed using the analysis of variance (ANOVA) at p≤0.05 followed by mean comparison analysis using the Duncan Multiple Range Test (DMRT) following using the Statistical Analysis System (SAS) software (Salleh et al., 2020b).

3. Results and Discussion

3.1 Germination Percentage

Standard germination test is the easiest technique to identify if the seeds will grow when being planted. Besides, germination test is the most reliable way of assessing seed viability (Davies et al., 2015). This is due to the controlled conditions which assist the seed to germinate. Based on there are many ways to perform the standard germination test such as germination using absorbent paper, between paper method, wet tissue and germination in sand (Hanson, 1985). The present study used wet tissue method to determine the rate of seed viability and vigour. Germination percentage, indicated rate of seed viability while, germination index and seedling vigour index indicated rate of seed vigour. According to seed can be labelled as germinated seed when the radical or plumule of seed are observed on the surface of seed (Qureshi et al., 2016). In this study, the successfully germinated chili seeds were observed based on the radical protrusion and the data were recorded and displayed using bar graph (Figure 1).

As shown in Figure 1, among all treatment, T1 (5 mg/L GA_3) recorded significantly higher germination percentage at 93% followed by T3 (15 mg/L GA_3) at 85% while T0 (control) and T2 (10 mg/L GA_3) showed statistically similar result of between 60% and 70%. GAs plays crucial role as natural regulator in processes involved seed germination which will stimulate the production of hydrolytic enzyme such as α-amylase in aleuron layer of the seed (Seo et al., 2009). Aleuron layer is the layer that surround thin-walled cells with starch grains in starchy endosperm. Thus, when GAs stimulates hydrolytic enzymes, the stored food in starchy endosperms are broken down into soluble sugars, amino acid, and other products which then transferred to growing embryo (Gupta and Chakrabarty, 2013). As a result, the transport of soluble sugar will promote growth and development of embryo and later induce seed germination through radicle protrusion. According to a study, exogenous GA_3 plays an important role in initiating the seed germination as it helps to counter balance the inhibition effect of abscisic acid (ABA) (Viera et al., 2002).

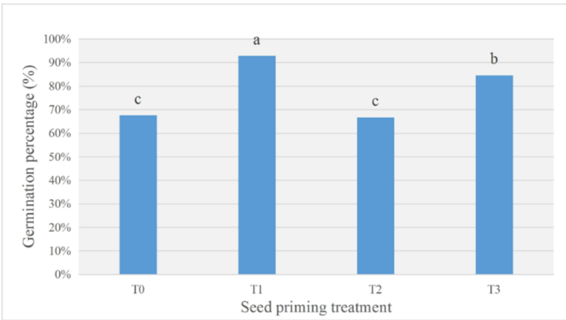

Figure 1: Effect of GA_3 on germination percentage of chili seeds. Different letters are significantly different based on the DMRT test at $p < 0.05$. T0: Control, T1: 5 mg/L GA_3, T2: 10 mg/L GA_3, and T3: 15 mg/L GA_3.

In this study, seed primed with 5 mg/L of GA_3 (T1) proven to have the highest germination percentage compared with untreated seeds (T0) and seeds which primed with higher concentration (T2: 10 mg/L, and T3: 15 mg/L, of GA_3). Thus, the germination of seeds was proven to be affected with exogenous application of GA_3 as it helps to reduce the seed dormancy. Other than that, the concentration of GA_3 also affect the germination percentage. This can be supported by the study carried out by in which three different concentrations of GA_3 were used as priming solvent for *Cyclamen africanum* which were 50, 100 and 150 mg/L of GA_3. As a result, seeds treated with 50 mg/L of GA_3 showed the highest germination percentage while 150 mg/L did not show any germination (Cornea-Cipcigan et al., 2020). As indicated by GA_3 showed higher significant effect at very low concentration which boost the germination process while too high concentration may suppress the process (Riley, 1987). Thus, lower concentration of GA_3 expressed higher seed germination as it able to boost germination process compared with seeds treated with higher concentration of GA_3.

3.2 Germination Index

Germination Index (GI) is the analysis technique that best describes the level of seed vigour. Seed vigour can be defined as the sum total of properties of seed which help to determine the activity level and performance of seed and seed lot during germination and also seedling emergence (Hampton, 1993). The GI was obtained by recording the number of germinated seeds on each day of the germination period. Higher GI values indicate higher and faster germination (Kader, 2005). As shown in Figure 2, T1 showed significantly higher GI at 117.23 while T0 and T2 recorded significantly lower values at 66.48 and 66.35, respectively.

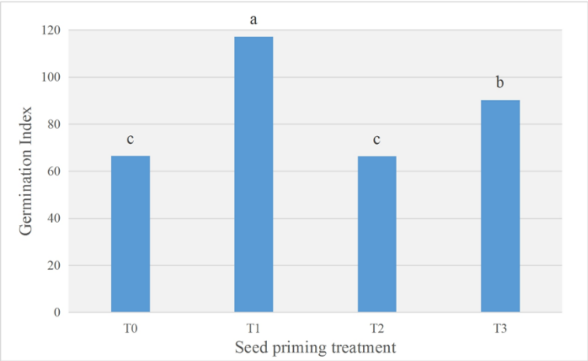

Figure 2: Effect of GA_3 on germination index of chili seeds. Different letters are significantly different based on the DMRT test at $p < 0.05$. T0: Control, T1: 5 mg/L GA_3, T2: 10 mg/L GA_3, and T3: 15 mg/L GA_3.

In general, T1 showed the highest GI among all treatment which indicate that this treatment able to express faster germination among all treatments. These results indicated that 5 mg/L of GA_3 not only improved the number of germinated seed (as indicated by the germination percentage), but also the speed of seed germination as indicated by the germination index. Seed germination process involved three stages mainly; 1) water imbibition by seed, 2) reactivation of metabolism, and 3) radical protrusion (Bewley ,1997). During the first stage (water imbibition by seed), the phytohormones play crucial role including gibberellic acid (GA_3). This phytohormone assist in diffusing aleurone layer and initiating signaling cascade which initiate the synthesis of α-amylase and other hydrolytic enzyme (Jacobsen and Chandler, 1987; Bethke et al., 1997). Hydrolytic enzyme present in the seed has a role in hydrolysis of starch, lipid, protein hemicellulose, polyphospahtes and other storage materials and turned it into simple available form for the embryo uptake which results in radical protrusion of the seed (Mayer and Poljakoff-Mayber, 1989; Salisbury and Ross, 1991). Hence, the increment of GA in the chili seeds during the seed priming treatment with GA_3 promote the synthesis of hydrolytic enzyme that involved in reactivation of metabolism for the germination process of the seeds in which increase the germination rate of chili seeds.

3.3 Seedling Vigour Index

Seedling vigour index symbolize the quality of seed which affects the establishment of field crop (Adebisi et al., 2012). Hence, the higher the seedling vigour index, the better the performance of the seed during early seedling growth. Result in Figure 3 indicated that T1 (12.73) and T3 (12.04) recorded statistically similar value for seedling vigour index but significantly higher compared to T0 and T2. In contrast, T0 recorded the lowest value at 6.92. Although T2 recorded slightly higher value at 8.51 as compared to T0, both treatments were statistically similar. Some researcher mentioned that early developmental stages for seedling need to be fueled with energy before it can become autotrophic (Mayer and Shain, 1974). The presence of phytohormones such as GA are significant to help the development of the seedling. Similar with germination percentage and germination rate, T1 (primed with 5 mg/L GA_3) showed the highest mean of seedling vigour index. According to a study, sufficient amount of GA_3 will be able to trigger the synthesis, activation and secretion of hydrolytic enzyme mentioned before in which promote the development of embryo of the seeds (Vieira et al., 2002). Hence, the 5 mg/L GA_3 could be regarded as an optimum amount of GA_3 treatment needed to promote the seedling vigour index of chili seeds.

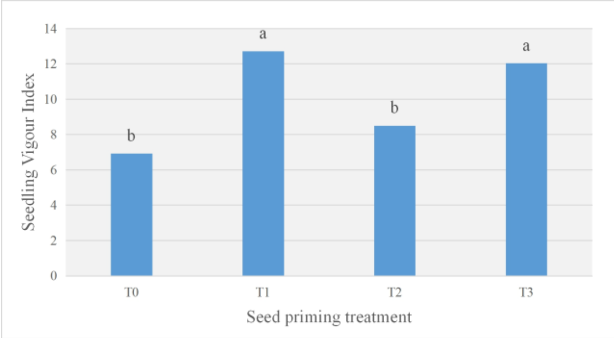

Figure 3: Seedling vigour index of treated chili seeds after 14 days of sowing.

Different letters are significantly different based on the DMRT test at $p < 0.05$. T0: Control, T1: 5 mg/L GA_3, T2: 10 mg/L GA_3, and T3: 15 mg/L GA_3.

3.4 Seedling Growth Performance of Primed Seeds

The seedling growth performance were recorded on the 7 and 14 days after sowing (DAS). Parameters recorded were seedling height and root length. Plant height was measured from the soil surface to the tip of the shoot. This parameter is one of essential indicator for the seedling growth as the growth can be observed visually. Based on Table 2, there are significant difference between seedlings treated with GA_3 and untreated seedlings when the seedlings were not watered for the duration of 7 days. The highest mean of height obtained during the severe water deficit stress was from T1 with a value of 2.11 cm. It was followed by T2 (1.77 cm), T3 (1.58), and lastly T0 (1.05 cm). Other than S5, there were no significant difference between the mean height of seedling except for S1 which only showed significant difference between treated seeds with the untreated one.

Table 2: Effect of seed priming treatment on height of seedling under water deficit treatment after 7 days of sowing.

Seed priming treatment	Water deficit treatment Height (cm)
T0	1.97b
T1	3.11a
S1	2.92a
T2	
T3	2.79a
T0	2.24a
T1	1.99a
S2	2.15a
T2	
T3	1.79a
T0	1.83a
T1	2.11a
S3	1.95a
T2	
T3	1.48a
T0	2.77a
T1	2.57a
S4	2.26a
T2	
T3	1.40a
T0	1.05c
T1	2.11a
S5	1.77ab
T2	
T3	1.58b

Note: Mean with different letters are significantly different based on DMRT test at p<

0.05. T0: Control, T1: 5 mg/L GA_3, T2: 10 mg/L GA_3, and T3: 15 mg/L GA_3. S1: Watering 8 times, S2: Watering 4 times, S3: Watering 2 times, S4: Watering 1 times, and S5: Not watered, in 2 weeks.

As shown in Figure 4, there were significant difference between water deficit treatments for each seed priming treatment after 14 days of sowing. For T0, S2 showed the highest significant mean of height at 5.76 cm while the lowest is S5 which at 0 cm. The pattern of T0 height was ascending between S1 and S2, but started to deplete in the treatment of S3, S4 and S5.

However, for treated seed (T1, T2 and T3), the highest significant mean of height were obtained by S1. The height of all treated seeds was in descending order from S1 to S5. T1 showed significant difference between water deficit treatment except for treatment between S2 and S3, and S3 and S4.

In contrast, T2 was proven to have significant difference between all water deficit treatments while T3 only showed no significant difference on S1 and S2. Akinbile mentioned that water application which is beyond the requirement amount would only result in losses and did not contribute in the increment of agronomic and yield of plant (Akinbile, 2010). It can be speculated that the decrease of height of T0 seeds that undergo S1 water deficit treatment is due to excess watering which cause excess moisture in the soil of the seedlings. As mentioned, young seedlings of *C. annum* unable to withstand the presence of deficit and excess of soil moisture, but older seedlings able to tolerate with the moisture stress to certain extent (Khan et al., 2012). Hence, the seedlings of T0 can be speculated as young seedlings while the treated seeds have advanced as older seedlings as GA_3 helps to boost their germination rate.

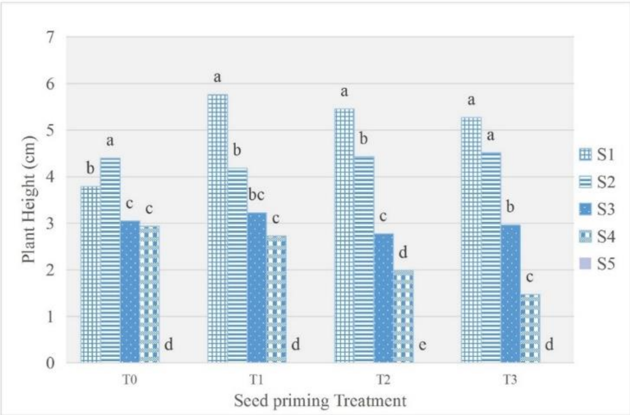

Figure 4: Effect of seed priming treatment on plant height of seedling under water deficit treatment after 14 days of sowing. Different letters are significantly different based on DMRT test at $p < 0.05$. T0: Control, T1: 5 mg/L GA_3, T2: 10 mg/L GA_3, and T3: 15 mg/L GA_3. S1: Watering 8 times, S2: Watering 4 times, S3: Watering 2 times, S4: Watering 1 times, and S5: Not watered, in 2 weeks.

In addition, the root length was measured from the soil line of seedling to the tip of the longest root observed from the seedling. This parameter was only recorded on 14 DAS as its involved disruptive sampling. In Figure 5, there were significant difference between water deficit treatments for each seed priming treatment after 14 days of sowing. For T0, S2 showed the highest significant mean of root length at 7.17 cm while the lowest is S5 which at 0 cm. The pattern of T0 height was ascending between S1 and S2, but started to deplete in the treatment of S3, S4 and S5. However, for treated seed (T1, T2 and T3), the highest significant mean of root length was obtained by S1. The root length was gradually decreasing under water deficit condition as the parameter were in descending order from S1 to S5.

T1 showed no significant difference between water deficit treatment except for treatment between S3 and S4, and S4 and S5. In contrast, T2 was proven also showed no significant difference between S3 and S4 while T3 only showed significant difference on S2 and S3, and S3 and S4. The water deficit stress level can be distinguished through the development of root of the seedlings. For untreated seed (T0), mild stress started to occur in S3 and S4 treatment in which only watered for 2 and 1 times only in 14 DAS, respectively. Then, severe stress occurs when the seedlings were not watered for the whole 2 weeks. Meanwhile, T1 (seed primed with 5 mg/L GA_3) undergo mild stress when watered once in 14 days and severe stress when there was no irrigation system applied for 14 days of observation.

The water stress level for T2 and T3 were similar with T1 as there were no significant difference when compared between seed priming treatments. Other than that, the results obtained for early seedling growth of chili under water deficit condition also assisted in determine the optimum watering times for chili and the start of water stress for each treatment. According to pattern of seedling growth, T0 undergone water stress during S1 treatment and only reached optimum amount of watering when watered according to S2 treatment in which watered 4 times for 14 DAS. Meanwhile, the optimum amount of watering of seed primed with GA_3 was S1 (8 times for 14 DAS) in which showed that the seedlings primed with GA_3 advanced earlier in term of growth when compared with seedling that acted as control sample.

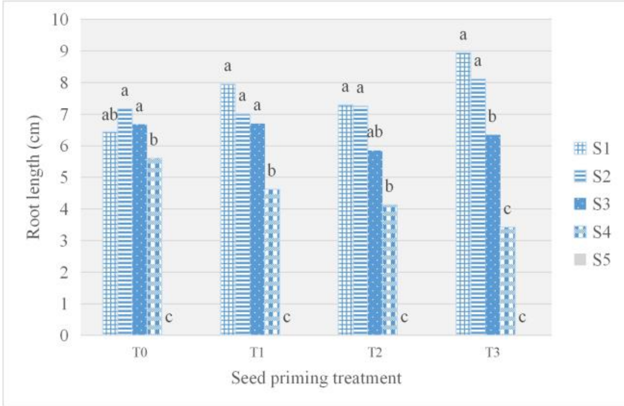

Figure 5: Effect of seed priming treatment on root length of seedling under water deficit treatment after 14 days of sowing. Different letters are significantly different based on DMRT test at $p < 0.05$. T0: Control, T1: 5 mg/L GA_3, T2: 10 mg/L GA_3, and T3: 15 mg/L GA_3. S1: Watering 8 times, S2: Watering 4 times, S3: Watering 2 times, S4: Watering 1 times, and S5: Not watered, in 2 weeks.

According to Kramer and Boyer the reduction of root length under low moisture content in soil did not show a big gap of difference when compared with shoot (Kramer and Boyer, 1995). Other than that, some researchers mentioned that the root elongation under low moisture content in soil is less inhibited than the development of shoot system and some species may even be promoted (Sharp and Davies, 1989). Thus, the root length of chili showed some decrement pattern but did not really affected during mild water deficit stress (S2 and S3).

A study also showed several species, such as maize, soybean and cotton, which maintain the rate of root elongation at water potential lower than -1.5 MPa, but the development started to be completely inhibited at higher water potentials (Spollen et al., 1993). Hence, severe water deficit stress (S4 and S5) affect the development of root towards chili seedling. According to some study, GA is determined to assist in stem elongation, response to abiotic stress, flowering and fruit development, but the GA treatment depends on species, concentration used and time of application (Cornea-Cipcigan et al., 2020). In overall, early seedling growth of T1 (seedlings primed with 5 mg/L of GA_3) was significantly higher compared to other treatments. Hence, it may be regarded as the optimum rate of GA_3 for seed priming of chili under water deficit condition.

4. CONCLUSION

Seed priming with GA_3 showed significant positive effects on the seed germination of chili seeds in accordance with its germination percentage, germination index and seedling vigour index. It also showed some significant positive effects in height and root length of early seedling growth under water stress. Then, the optimum rate of GA_3 which showed better seed germination, and early seedling growth of chili under water stress was chili seeds that was treated with 5mg/L of GA_3(T1). Moreover, the water deficit stress level was also determined. S1 and S2 treatment can be considered as non-stress condition while S3 and S4 as mild to moderate stress and S5 as severe stress. Furthermore, S2 was considered as optimum watering treatment and S1 was labeled as mild water stress due to excess amount of water applied which reduced the growth of untreated chili seeds (T0). For further study, it could be suggested that the experiment be repeated using other types of plant species to determine the consistency of the positive effects of seed priming using GA_3 in improving seed germination and early seedling growth of plants under water deficit condition.

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